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## Article

# Effect of Ecotype and Environment on Oil Content, Fatty Acid, and Sterol Composition of Seed, Kernel, and Epicarp of the Atlas Pistachio

Amina Labdelli <sup>1</sup> , Mohammed Tahirine <sup>1</sup> , Abdelhamid Foughalia <sup>1</sup>, Kamel Zemour <sup>2,3</sup>, Muriel Cerny <sup>3</sup>, Ahmed Adda <sup>2</sup>, Valérie Simon <sup>3</sup> and Othmane Merah <sup>3,4,\*</sup>

<sup>1</sup> Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biskra 07000, Algeria

<sup>2</sup> Laboratory of Agro-Biotechnology and Nutrition in Semi-Arid Areas, Ibn Khaldoun University, Tiaret 14000, Algeria

<sup>3</sup> Laboratoire de Chimie Agro-Industrielle (LCA), Université de Toulouse, INRA, INPT, 31030 Toulouse, France

<sup>4</sup> Département Génie Biologique, IUT A, Université Paul Sabatier, 32000 Auch, France

\* Correspondence: othmane.merah@ensiacet.fr or othmane.merah@inp-toulouse.fr; Tel.: +33-53-4323523

**Abstract:** The Atlas pistachio fruits are an important source of food due to their high fat content and medicinal interest in arid lands. For a better use, it is necessary to understand the diversity and distribution of lipids in their fruit. The purpose of the present study is to determine oil content, fatty acid, and phytosterol composition using gas chromatography of three ecotypes in Algeria during two consecutive years. The seed oil contents ranged from 32 to 47.8% depending on both year and ecotype. Unsaturated fatty acids (FA) represent at least 71% of total FA. The fruit contains a considerable level of phytosterols. The highest level of oil content and unsaturated FA were observed in the fruit of Bechar regardless of the part of the fruit. The study indicated that oil quality of the Atlas pistachio seed varies according to genotype and environmental conditions, and the findings could help breeders for diversity and selection program management.

**Keywords:** Atlas pistachio; oil content; fatty acids; phytosterol; environment



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## 1. Introduction

Atlas pistachio (*Pistacia atlantica* Desf. subsp. *atlantica*) is an extremely significant species for rural communities. It is known for both its pharmacological properties and traditional usage. The various parts of the Atlas pistachio, including the resin, leaves, fruits and galls, have been used for a long time in diverse regions as efficient remedies for a wide range of diseases. For instance, leaves, gull, and gum resin have a wound-healing effect and have been used to treat digestive and gastrointestinal disorders, diabetes, Alzheimer, hepatic, kidney, and brain diseases [1–4]. The Atlas pistachio's fruits are also a very abundant source of bioactive compounds [5,6]. They have aphrodisiac properties and can be used to treat respiratory problems and cardiovascular disease. Furthermore, due to their richness in oleic and linolenic acids and phytosterols, especially  $\beta$ -sitosterol, the fruits of *P. atlantica* Desf. have been recommended as a potential source for producing vegetable oils [7].

In fact, research in recent decades has concentrated on enhancing the role of natural food consumption in the improvement of human health.

The development of functional, therapeutic, and medicinal foods appears to have a key role in current dietary habits [8,9]. For example, fatty acids are essential in diets because they provide energy and fat-soluble vitamins. They also help to structure cell membranes by associating with glycerides [10]. Moreover, due to their effects on low-density lipoprotein cholesterol (LDL) concentration and serum cholesterol, some fatty acids have been shown to either protect against or cause atherosclerosis and coronary thrombosis [11]. Generally, Western diets are deficient in omega-3 fatty acids and rich in omega-6 fatty acids. The intake of vegetable oils rich in polyunsaturated fatty acids (PUFA) is the main reason

for this deficiency [12,13]. At present, industrial and modern agriculture has led to an increase in the consumption of omega-6-rich and omega-3-poor foods [13]. In France, for example, the ANSES (Agence française de sécurité sanitaire des aliments) estimates that the omega-6/omega-3 ratio should not exceed 5, but the gauged ratio for Western food is generally higher than 10 [14].

Indeed, the phytosterols have an undeniable place in foods, cosmetics, and herbal medicine applications [15]. Consequently, they are engineered and have been marketed as being able to reduce total plasma cholesterol and LDL and minimize cardiovascular risks, resulting in a reduction in blood cholesterol. Thus, they possess anti-inflammatory, antifungal, anti-ulcerative, antibacterial, antitumor, and anti-cancer activities [16,17].

In the literature regarding plants, the quality of its edible oils depends on its genotype, growing environmental conditions [18], and its fatty acid [19,20] and phytosterols composition [21].

In recent years, a great number of studies have focused on studying the quality of fresh fruit composition in an appropriate way. For the Atlas pistachio, kernel and epicarp are biological parts of the seed. The epicarp is a thin layer adhering to the kernel that induces the dormancy of the seed. However, the whole seed is crushed and consumed by the local population for many uses [22]. Therefore, this paper aimed to study the oil content, phytosterols, and fatty acids composition in seed, kernel, and epicarp of the Atlas pistachio. In addition, this research evaluated the effect of the genotype and environmental conditions on these components.

This contribution is an attempt to highlight the possibility of using the Atlas pistachio fruits as raw material sources of healthy vegetable oil for human intake.

## 2. Materials and Methods

### 2.1. Plant Materials

Seeds from three separate populations of Atlas pistachio growing widely in different regions of Algeria (semi-arid (Batna), arid (Djelfa), and hyper-arid (Bechar)) were used in the present study. These regions are distinguished by their various geographical locations and their environmental characteristics (Table 1). The main objective was, therefore, to determine the quality of the different seeds over two years, 2015 and 2016.

The seeds were collected from the beginning of September to the end of October for each year (2015 and 2016) and deposited in the herbarium of the Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biskra, Algeria (006CRSTRA0002). It should be emphasized that in 2016, the seeds harvested from the region of Batna were damaged.

All the samples were stored at 4 °C until analysis [23]. The epicarp was manually separated from the seeds on the same day of analysis.

**Table 1.** Main Climatic Characteristics of the Atlas Pistachio Collection Sites for Two Years (2015 and 2016).

Location	Annual Parameters							
	Rainfall (mm)		Temperature (°C)					
			Mean		Min		Max	
	2015	2016	2015	2016	2015	2016	2015	2016
Bechar (A)	72	111	22.1	22.3	15.5	15.2	28.3	28.9
Batna (B)	341	210	15.4	16	7.8	8.3	23.2	24.1
Djelfa (D)	279	185	15.1	15.4	8.9	9.7	21.2	21.2

Source: National Meteorology Office of Algeria (ONM).

### 2.2. Biochemical Analyses

#### 2.2.1. Oil Extraction

Using a Soxhlet glassware (Fischer, Illkirch, France) and 200 mL of cyclohexane (Merck, Lyon, France), the crude oil of the seeds was extracted from 10 g of milled fine powder (NF

EN ISO 659). The extraction was carried out during six hours according to Zemour et al. [18] with some modifications. After the extraction, the obtained oil was then concentrated by evaporating the solvent using a rotary evaporator (IK HB 10 digital, Keison, Chelmsford, UK) at 50 °C and under reduced pressure. The concentrated oil was then stored at 4 °C.

#### 2.2.2. Fatty Acid Composition of the Different Parts of the Seed

Fatty acid methyl esters (FAME: ISO 12966-3, 2016) were analyzed by using GC-FID 3900 gas chromatograph (Varian 3900, Palo Alto, Milpitas, CA, USA) containing a CP-select CB capillary column (50 m × 0.25 mm × 0.25 µm) and equipped with a flame ionization detector (FID). The seed powders of both kernel and epicarp were obtained using a coffee grinder. Then, 400 mg of each powder was solubilized in 5 mL of Tert-butyl methyl ether (TBME; ME0552, Scharlab, Barcelona, Spain). After agitation and filtration, 100 were taken from the obtained solution and then transferred to a suitable insert. The transesterification reaction was carried out by adding 50 µ of 0.2 M Trimethyl sulfonium hydroxide (TMSH) (Macherey-Nagel, Hoerd, France). The analysis was then performed using the following operating conditions: the initial oven temperature was 185 °C for 40 min, then raised to 250 °C with a frequency of 15 °C/min and maintained at 250 °C for 10 min; flow rate of the carrier gas (helium) was 1.2 mL/min, split ratio 1:100. The temperature of injector and detector was set to 250 °C [5,24]. Fatty acids profile was determined by comparing the retention times with an external standard mixture of rapeseed oil (Supelco, Bellefonte, PA, USA).

#### 2.2.3. Oxidizability Value (Cox)

On the basis of the percentage of C18 unsaturated fatty acids (oleic, linoleic, and linolenic acids), the Cox value of the seed, kernel, and epicarp was calculated, following the formula proposed by Fatemi and Hammond [25]:

$$\text{Cox Value} = \frac{(1 \times (18 : 1 \%) + 10.3 \times (18 : 2 \%) + 21.6 \times (18 : 3 \%))}{100} \quad (1)$$

#### 2.2.4. Extraction and Determination of Phytosterols

The extraction of phytosterols was carried out according to the standard method NF 5508 described by Roche et al. [24].

In order to determine the sterol contents, 500 mg of each extract was mixed with 4 mL of ethanolic KOH (1 M). The obtained solution was added to a tube containing 50 µL of an internal standard (cholestanol (dihydrocholesterol; Aldrich Chemicals Co., Lyon, France)). After shaking and heating the mixture in a water bath at 60 °C for 60 min, 1 mL of distilled water and 6 mL of iso-hexane were added. After decantation and recovering of sterol compounds, a step of silylation was performed by addition of 40 µL N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (Restek, Lisses, France). Finally, the sterol composition was determined using a Perkin–Elmer gas chromatograph equipped with a CP-SIL 5CB column (30 m (i.d.: 0.25 mm)), an on-column injector, and a flame ionization detector (FID). Internal analysis conditions were the following: 365 °C for the detector temperature and 160 until 350 °C for the injector temperature.

### 2.3. Statistical Analysis

All reported data were expressed as means ± standard deviation (three repetitions). A two-way analysis of variance (ANOVA) with Duncan post hoc tests was performed using STATISTICA software package (StatSof, Tulsa, OK, USA). Differences between means at 5%, 1%, and 0.1% levels ( $p < 0.05$ , 0.01, or 0.001, respectively) were considered statistically significant.

## 3. Results

### 3.1. Oil Content

The result indicated that the biochemical composition of oil of Atlas pistachio fruits varied significantly ( $p \leq 0.001$ ) depending on the bioclimatic stage (Table 2).

**Table 2.** Fatty Acids (%) Composition and Oil Content (%) of Seeds of Atlas Pistachio in Three Geographic Zones in Algeria for Two Years (2015 and 2016).

Fatty Acid (%) Seed	Bechar		Batna		Djelfa		F-Test
	2015	2016	2015	2016	2015	2016	
Oil content (%)	47.8 <sup>d</sup>	37.47 ± 2.02 <sup>c</sup>	32 <sup>b</sup>	-	40.1 <sup>a</sup>	40.4 ± 2.16 <sup>a</sup>	55.81 ***
Saturated fatty acid (SFA)							
C16:0 (Palmitic acid)	20.36 ± 0.04 <sup>b</sup>	22.87 ± 0.21 <sup>b</sup>	23.25 ± 0.06 <sup>b</sup>	-	24.01 ± 0.08 <sup>a</sup>	26.65 ± 0.74 <sup>a</sup>	128.66 ***
C18:0 (Stearic acid)	2.26 ± 0.01 <sup>a</sup>	1.71 ± 0.01 <sup>b</sup>	1.68 ± 0.01 <sup>b</sup>	-	1.8 ± 0.01 <sup>b</sup>	2.07 ± 0.02 <sup>a</sup>	1489.9 ***
C20:0 (Arachidic acid)	0.16 ± 0.01 <sup>a</sup>	0.09 ± 0.08 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>	-	0.15 <sup>a</sup>	0.06 ± 0.11 <sup>a</sup>	1.5 ns
Total SFA	22.78 ± 0.06 <sup>c</sup>	24.67 ± 0.29 <sup>b</sup>	25.08 ± 0.08 <sup>b</sup>	-	25.96 ± 0.09 <sup>b</sup>	28.79 ± 0.86 <sup>a</sup>	101.01 ***
Monounsaturated fatty acid (MUFA)							
C16:1n7 (Palmitoleic acid)	0.78 ± 0.01 <sup>e</sup>	1.17 ± 0.02 <sup>b</sup>	0.85 <sup>d</sup>	-	1.99 ± 0.02 <sup>a</sup>	0.98 <sup>c</sup>	7274.4 ***
C18:1n9 (Oleic acid)	52.03 ± 0.06 <sup>a</sup>	47.66 ± 0.14 <sup>b</sup>	43.29 ± 0.08 <sup>c</sup>	-	37.35 ± 0.16 <sup>e</sup>	40.94 ± 0.17 <sup>d</sup>	5846 ***
C18:1n7 (Vaccenic acid)	1.15 ± 0.01 <sup>d</sup>	1.48 ± 0.01 <sup>b</sup>	1.04 ± 0.01 <sup>e</sup>	-	1.77 ± 0.01 <sup>a</sup>	1.21 ± 0.03 <sup>c</sup>	1413.1 ***
C20:1n9 (Eicosenoic acid)	0.26 ± 0.01 <sup>a</sup>	0.19 ± 0.02 <sup>bc</sup>	0.13 ± 0.01 <sup>d</sup>	-	0.17 ± 0.03 <sup>c</sup>	0.21 ± 0.02 <sup>b</sup>	23.72 ***
Total MUFA	54.22 ± 0.08 <sup>a</sup>	50.51 ± 0.18 <sup>b</sup>	45.31 ± 0.11 <sup>c</sup>	-	41.29 ± 0.21 <sup>e</sup>	43.43 ± 0.21 <sup>d</sup>	4585 ***
Polyunsaturated fatty acid (PUFA)							
C18:2n6 (Linoleic acid)	22.30 ± 0.02 <sup>e</sup>	23.92 <sup>d</sup>	28.85 ± 0.06 <sup>b</sup>	-	31.14 ± 0.16 <sup>a</sup>	26.78 ± 0.66 <sup>c</sup>	419.9 ***
C18:3n3 (Linolenic acid)	0.71 ± 0.01 <sup>e</sup>	0.89 ± 0.01 <sup>c</sup>	0.75 ± 0.01 <sup>d</sup>	-	1.24 ± 0.02 <sup>a</sup>	1.09 ± 0.01 <sup>b</sup>	1037.5 ***
Total PUFA	23.01 ± 0.03 <sup>e</sup>	24.81 ± 0.01 <sup>d</sup>	29.61 ± 0.06 <sup>b</sup>	-	32.38 ± 0.18 <sup>a</sup>	27.87 ± 0.67 <sup>c</sup>	447 ***
Total MUFA + PUFA	77.23 ± 0.11 <sup>a</sup>	75.32 ± 0.19 <sup>b</sup>	74.92 ± 0.17 <sup>b</sup>	-	73.67 ± 0.39 <sup>c</sup>	71.3 ± 0.88 <sup>d</sup>	88.8 ***
(MUFA + PUFA)/SFA	3.39	3.05	2.99	-	2.84	2.48	-
PUFA/SFA	1.01	1	1.18	-	1.24	0.96	-
Omega-9/Omega-6	2.33 <sup>a</sup>	1.99 ± 0.01 <sup>b</sup>	1.50 ± 0.01 <sup>d</sup>	-	1.2 <sup>e</sup>	1.53 ± 0.03 <sup>c</sup>	2690 ***
Omega-6/Omega-3	31.26 ± 0.27 <sup>b</sup>	26.78 ± 0.35 <sup>c</sup>	38.3 ± 0.28 <sup>a</sup>	-	25.05 ± 0.31 <sup>d</sup>	24.57 ± 0.62 <sup>d</sup>	648.9 ***
Cox value	2.97 <sup>e</sup>	3.13 <sup>d</sup>	3.57 ± 0.01 <sup>b</sup>	-	3.85 ± 0.02 <sup>a</sup>	3.40 ± 0.07 <sup>c</sup>	339.5 ***

Values (means ± SD; n = 3). Within each line, lowercase letters indicate significance at  $p < 0.05$  probability level according to Duncan tests. \*\*\* denote significant at 0.001 probability, respectively. ns = non-significant.

The averaged data (Table 2) showed that the oil content fluctuates between 32 and 47.8%, recorded by Batna and Bechar seeds, respectively. However, this content decreased from 2015 to 2016 (approximately –10%) in Bechar seeds, while it remained stable in the Djelfa ecotype (Table 2).

### 3.2. Fatty Acid Composition and Oxidizability Value (Cox)

There were significant differences among fatty acids (FAs) in epicarp, kernel, and seed (Tables 2–4) ( $p \leq 0.001$ ). This difference among ecotypes is often due to both genetic and environmental factors. The different parts of fruits indicated large variations in their FA category (SFA, MUFA, and PUFA) in 2015 and 2016.

Among the saturated fatty acids (SFAs), palmitic acid (C16:0) was predominant in seeds, kernel, and epicarp, followed by stearic acid (C18:0). On the other hand, arachidic acid was present only in traces (C20:0). The findings have also indicated that the oleic (C18:1n9) and linoleic (C18:2n6) acids are the predominant monounsaturated fatty acids in the fruits (Tables 2–4; Figure 1).

**Table 3.** Fatty Acid (%) Composition of the Kernel of Atlas Pistachio in Three Geographic Zones in Algeria for Two Years (2015–2016).

Fatty Acid (%) Kernel	Bechar		Batna		Djelfa		F-Test
	2015	2016	2015	2016	2015	2016	
Saturated fatty acid (SFA)							
C16:0 (Palmitic acid)	14.44 ± 0.08 <sup>d</sup>	17.18 ± 0.07 <sup>b</sup>	15.60 ± 0.09 <sup>c</sup>	-	15.59 ± 0.15 <sup>c</sup>	18.07 ± 0.19 <sup>a</sup>	396.4 ***
C18:0 (Stearic acid)	2.90 ± 0.02 <sup>a</sup>	1.83 ± 0.01 <sup>e</sup>	2.02 ± 0.01 <sup>d</sup>	-	2.35 ± 0.02 <sup>c</sup>	2.51 ± 0.03 <sup>b</sup>	1652 ***
C20:0 (Arachidic acid)	0	0.13	0	-	0	0.20	-
Total SFA	17.34 ± 0.10 <sup>e</sup>	19.13 ± 0.07 <sup>b</sup>	17.62 ± 0.10 <sup>d</sup>	-	17.94 ± 0.17 <sup>c</sup>	20.78 ± 0.22 <sup>a</sup>	485.4 ***

Table 3. Cont.

Fatty Acid (%) Kernel	Bechar		Batna		Djelfa		F-Test
	2015	2016	2015	2016	2015	2016	
Monounsaturated fatty acid (MUFA)							
C16:1n7 (Palmitoleic acid)	0.54 ± 0.01 <sup>d</sup>	0.86 <sup>b</sup>	0.6 <sup>c</sup>	-	0.91 ± 0.01 <sup>a</sup>	0.59 ± 0.02 <sup>c</sup>	1097 ***
C18:1n9 (Oleic acid)	52.88 ± 0.06 <sup>b</sup>	50.41 ± 0.04 <sup>c</sup>	53.22 ± 0.05 <sup>a</sup>	-	48.86 ± 0.09 <sup>e</sup>	46.88 ± 0.18 <sup>d</sup>	2216 ***
C18:1n7 (Vaccenic acid)	0.98 ± 0.01 <sup>d</sup>	1.34 ± 0.01 <sup>a</sup>	0.95 ± 0.01 <sup>a</sup>	-	1.15 ± 0.01 <sup>b</sup>	0.99 ± 0.01 <sup>c</sup>	1715 ***
C20:1n9 (Eicosenoic acid)	0 <sup>b</sup>	0 <sup>b</sup>	0.24 ± 0.01 <sup>a</sup>	-	0 <sup>b</sup>	0.26 ± 0.03 <sup>a</sup>	252.3 ***
Total MUFA	54.40 ± 0.07 <sup>b</sup>	52.61 ± 0.05 <sup>c</sup>	55 ± 0.06 <sup>a</sup>	-	50.92 ± 0.1 <sup>d</sup>	48.73 ± 0.24 <sup>e</sup>	3233 ***
Polyunsaturated fatty acid (PUFA)							
C18:2n6 (Linoleic acid)	27.71 ± 0.05 <sup>c</sup>	27.61 ± 0.06 <sup>d</sup>	26.83 ± 0.03 <sup>e</sup>	-	30.52 ± 0.04 <sup>a</sup>	29.76 ± 0.05 <sup>b</sup>	3593 ***
C18:3n3 (Linolenic acid)	0.56 ± 0.01 <sup>c</sup>	0.64 ± 0.01 <sup>b</sup>	0.52 ± 0.04 <sup>d</sup>	-	0.62 ± 0.05 <sup>b</sup>	0.73 ± 0.02 <sup>a</sup>	22.33 ***
Total PUFA	28.27 ± 0.05 <sup>c</sup>	28.25 ± 0.06 <sup>c</sup>	27.35 ± 0.07 <sup>d</sup>	-	31.14 ± 0.09 <sup>a</sup>	30.49 ± 0.07 <sup>b</sup>	2888 ***
Total MUFA + PUFA	82.67 ± 0.12 <sup>a</sup>	80.86 ± 0.11 <sup>d</sup>	82.35 ± 0.13 <sup>b</sup>	-	82.06 ± 0.19 <sup>c</sup>	79.22 ± 0.31 <sup>e</sup>	486 ***
(MUFA + PUFA)/SFA	4.74	4.23	4.67	-	4.57	3.81	-
PUFA/SFA	1.63	1.47	1.55	-	1.73	1.46	-
Omega-9/Omega-6	1.91 <sup>b</sup>	1.83 <sup>c</sup>	1.98 <sup>a</sup>	-	1.60 <sup>d</sup>	1.58 <sup>e</sup>	7849 ***
Omega-6/Omega-3	49.78 ± 0.43 <sup>a</sup>	43.37 ± 0.31 <sup>b</sup>	51.45 ± 3.9 <sup>a</sup>	-	49.16 ± 3.85 <sup>a</sup>	40.98 ± 1.22 <sup>b</sup>	9.609 **
Cox Value	3.50 ± 0.01 <sup>c</sup>	3.49 ± 0.01 <sup>d</sup>	3.41 ± 0.01 <sup>e</sup>	-	3.77 ± 0.01 <sup>a</sup>	3.69 <sup>b</sup>	1079 ***

Values (means ± SD; n = 3). Within each line, lowercase letters indicate significance at  $p < 0.05$  probability level according to Duncan tests. \*\* and \*\*\* denote significant at 0.01 and 0.001 probability, respectively.

Table 4. Fatty Acid (%) Composition of the Epicarp of Atlas Pistachio in Three Geographic Zones in Algeria for Two Years (2015–2016).

Fatty Acid (%) Epicarp	Bechar		Batna		Djelfa		F-Test
	2015	2016	2015	2016	2015	2016	
Saturated fatty acid (SFA)							
C16:0 (Palmitic acid)	28.05 ± 0.03 <sup>e</sup>	30.21 ± 0.24 <sup>c</sup>	29.27 ± 0.06 <sup>d</sup>	-	31.50 ± 0.06 <sup>b</sup>	34.81 ± 0.10 <sup>a</sup>	994.2 ***
C18:0 (Stearic acid)	1.60 <sup>a</sup>	1.61 ± 0.06 <sup>a</sup>	1.32 ± 0.01 <sup>c</sup>	-	1.48 ± 0.02 <sup>b</sup>	1.69 ± 0.0 <sup>a</sup>	73.03 ***
Total SFA	29.65 ± 0.03 <sup>e</sup>	31.82 ± 0.30 <sup>c</sup>	30.59 ± 0.06 <sup>d</sup>	-	32.99 ± 0.07 <sup>b</sup>	36.50 <sup>a</sup>	1139 ***
Monounsaturated fatty acid (MUFA)							
C16:1n7 (Palmitoleic acid)	1.09 ± 0.01 <sup>d</sup>	1.45 ± 0.03 <sup>b</sup>	1.08 <sup>d</sup>	-	2.45 ± 0.02 <sup>a</sup>	1.17 ± 0.02 <sup>c</sup>	1905 ***
C18:1n9 (Oleic acid)	51.45 ± 0.01 <sup>a</sup>	47.67 ± 0.04 <sup>b</sup>	36.28 ± 0.03 <sup>d</sup>	-	31.72 ± 0.12 <sup>e</sup>	37.01 ± 0.08 <sup>c</sup>	42,006 ***
C18:1n7 (Vaccenic acid)	1.43 <sup>c</sup>	1.8 ± 0.04 <sup>b</sup>	1.19 ± 0.01 <sup>d</sup>	-	2.02 ± 0.01 <sup>a</sup>	1.19 <sup>d</sup>	1443 ***
Total MUFA	53.97 ± 0.02 <sup>a</sup>	50.92 ± 0.11 <sup>b</sup>	38.55 ± 0.03 <sup>d</sup>	-	36.20 ± 0.16 <sup>e</sup>	39.37 ± 0.1 <sup>c</sup>	55,626 ***
Polyunsaturated fatty acid (PUFA)							
C18:2n6 (Linoleic acid)	15.57 ± 0.0 <sup>e</sup>	17.27 ± 0.27 <sup>d</sup>	29.96 ± 0.06 <sup>a</sup>	-	29.36 ± 0.07 <sup>b</sup>	23.0 ± 0.04 <sup>c</sup>	8310 ***
C18:3n3 (Linolenic acid)	0.83 ± 0.03 <sup>d</sup>	0 <sup>e</sup>	0.89 ± 0.01 <sup>c</sup>	-	1.46 ± 0.03 <sup>a</sup>	1.12 ± 0.01 <sup>b</sup>	2814 ***
Total PUFA	16.4 ± 0.03 <sup>d</sup>	17.27 ± 0.27 <sup>c</sup>	30.86 ± 0.07 <sup>a</sup>	-	30.82 ± 0.09 <sup>a</sup>	24.12 ± 0.05 <sup>b</sup>	8691 ***
Total MUFA + PUFA	70.37 ± 0.05 <sup>a</sup>	68.19 ± 0.38 <sup>c</sup>	69.41 ± 0.1 <sup>b</sup>	-	67.02 ± 0.25 <sup>d</sup>	63.5 ± 0.15 <sup>e</sup>	1078 ***
(MUFA + PUFA)/SFA	2.37	2.14	2.27	-	2.03	1.74	-
PUFA/SFA	0.55	0.54	1	-	0.93	0.66	-
Omega-9/Omega-6	3.31 <sup>a</sup>	2.76 ± 0.04 <sup>b</sup>	1.21 <sup>d</sup>	-	1.08 ± 0.01 <sup>e</sup>	1.61 <sup>c</sup>	8019 ***
Omega-6/Omega-3	18.88 ± 0.57 <sup>c</sup>	-	33.55 ± 0.49 <sup>a</sup>	-	20.12 ± 0.32 <sup>b</sup>	20.54 ± 0.15 <sup>b</sup>	824.8 ***
Cox Value	2.30 ± 0.01 <sup>c</sup>	2.26 ± 0.03 <sup>d</sup>	3.64 <sup>a</sup>	-	3.66 ± 0.01 <sup>a</sup>	2.98 ± 0.01 <sup>b</sup>	7157 ***

Values (means ± SD; n = 3). Within each line, lowercase letters indicate significance at  $p < 0.05$  probability level according to Duncan tests. \*\*\* denotes significant at 0.001.

In fact, the high content of linoleic acid has a significant nutritional aspect, because it is a precursor of essential fatty acids (EFA), along with linolenic acid (C18:3n-3).

The obtained findings suggest that the seed, kernel, and epicarp are also excellent sources of oleic, linoleic, and palmitic acids (Tables 2–4; Figure 1), while palmitoleic, stearic, and linolenic acids were found in lower amounts in all analyzed samples. Traces of arachidic and eicosenoic acids were detected in all samples.



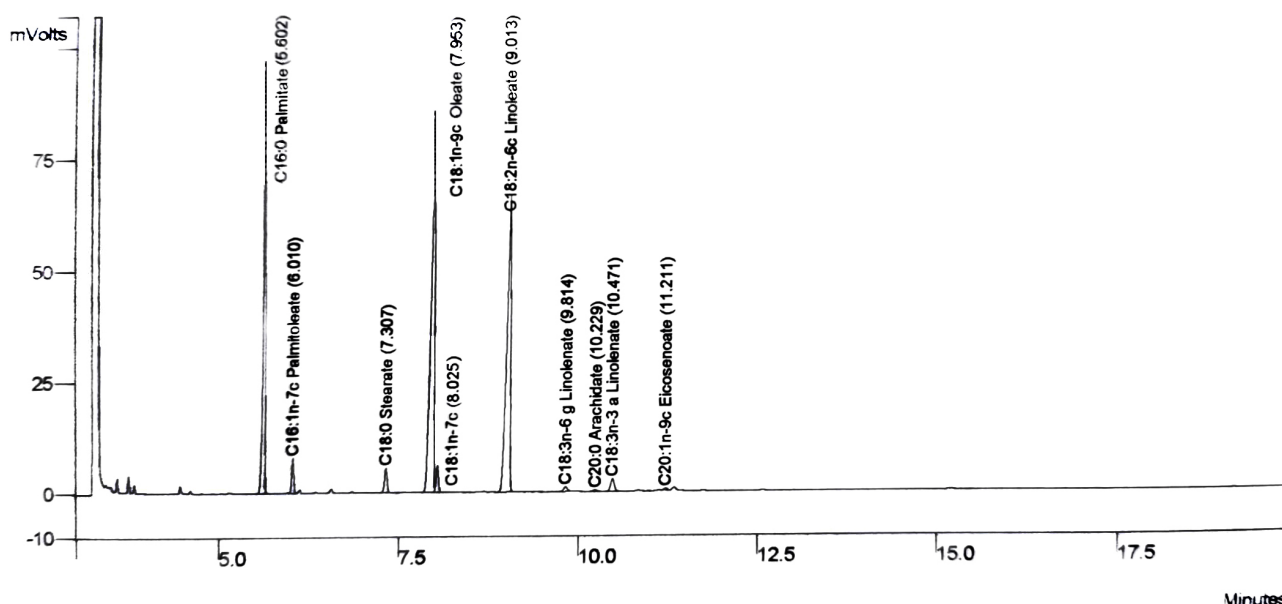


Figure 1. Chromatogram of Fatty Acids from Atlas Pistachio Seeds.

In the Bechar ecotype, the content of saturated (SFA) and polyunsaturated (PUFA) fatty acids in seeds, kernel, and epicarp increased by 2% from 2015 to 2016, but this content decreased in Djelfa by 3% in 2016. For all parties of the fruit and from 2015 to 2016, the content of monounsaturated fatty acids (MUFA) decreased by approximately 3% in Bechar and increased by 2% in the Djelfa ecotypes (Tables 2–4).

In general, it has been shown that seeds from the Djelfa and Batna regions have higher PUFAs contents than fruits from the Bechar region ( $p < 0.05$ ). In all three parts, oleic acid is the most abundant. In fact, the kernel is the part richest in oleic acid (50.4%), followed by the seeds (44.2%) and then the epicarp (40.8%). The same applies to linoleic acid. However, the palmitic acid is present mainly in the epicarp (30.8%), followed by the seed (23.4%) and then the kernel (16.2%) (Tables 2–4).

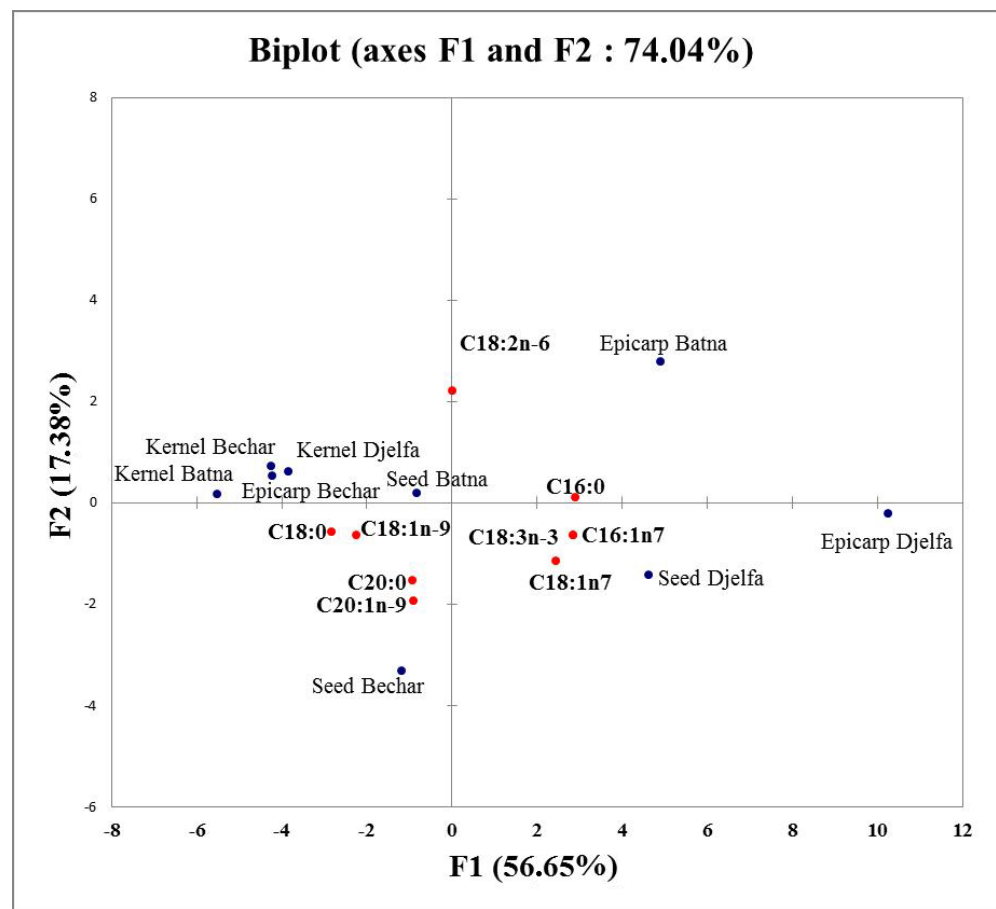
As shown in Tables 2 and 3, the kernel contained the highest ratio of unsaturated fatty acids to saturated fatty acids (PUFA + MUFA)/SFA with approximately 1.4 and 2.1 times more than seed and epicarp, respectively.

Moreover, on the basis of the results mentioned above, it was revealed that the ratio of PUFA/SFA in seed (~1) (Table 2) and kernel (means 1.5) (Table 3) was the highest, followed by epicarp (0.5–1) (Table 4), indicating significant differences among the three parts of seeds and ecotypes.

The Cox value varies in the range of 2.9–3.8 in seed (Table 2), 3.4–3.7 in the kernel (Table 3), and 2.2–3.6 in the epicarp of all ecotypes (Table 4).

### 3.3. Data Analysis by Principal Component Analysis (PCA)

According to the principal components analysis, 56.65% of the total variance was explained by the first principal component F1 and 17.38% by the second principal component F2. In total, the two principal components F1 and F2 were able to explain 74.04% of total variance. The PCA results also revealed significant variation in the fatty acids content of the different sections of Atlas pistachio fruits (Figure 2). Indeed, in the means of two years, 2015 and 2016, a clear separation between the seed, kernel, and epicarp could be observed. However, the epicarp of the three ecotypes was characterized by high levels of C16:0, C16:1n7, and C18:1n7, while the kernel was characterized mainly by higher values of C18:0 and C18:1n9. In addition, C18:3n3, C18:3n3, C20:0, and C20:1n9 acids were the main acids that constituted the studied seed (Figure 2).



**Figure 2.** Two-dimensional plot on axes F1 and F2 of the fatty acids content in different parts of Atlas pistachio fruits during two years using PCA.

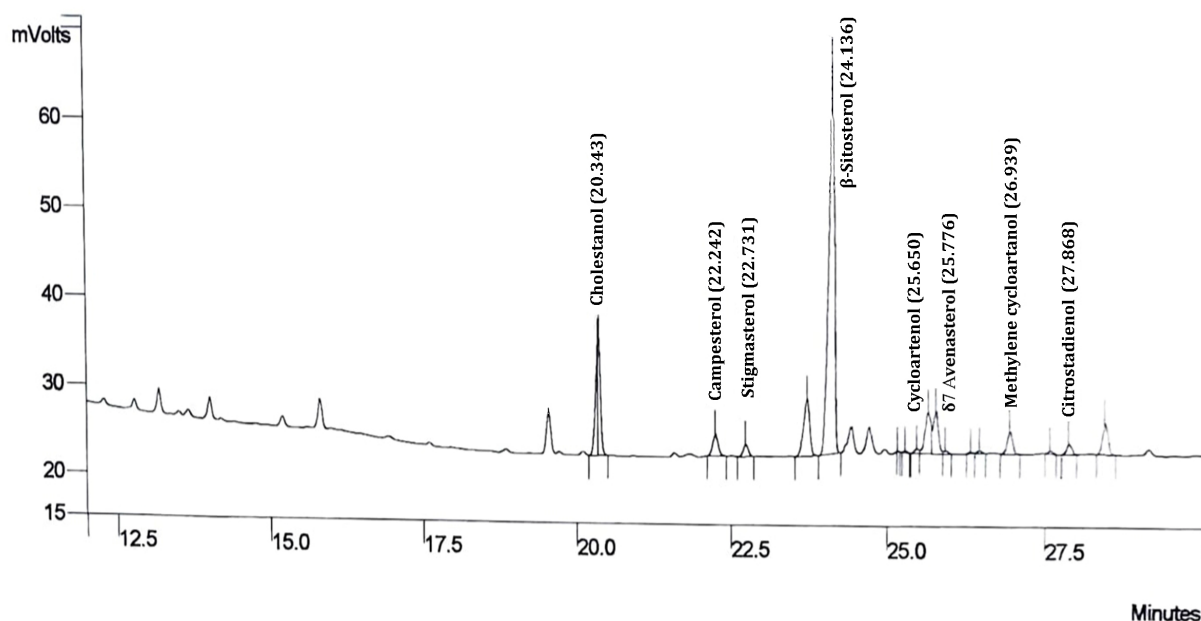
### 3.4. Determination of Phytosterols

The present study indicated that Djelfa fruits have a higher concentration of total phytosterols than the Bechar ecotype (Table 5 and Figure 3). Indeed, from 2015 to 2016, the total phytosterols content decreased in the Djelfa and Bechar ecotypes. Furthermore, the findings indicated that the  $\beta$ -sitosterol was higher in all the ecotypes among the demethylphytosterols (DMS), and the DMS was not stable over the years (Table 5). Among all the analyzed samples, the ecotype of Bechar (2015) showed the highest content of  $\beta$ -sitosterol (81.4 mg/100 g), while the ecotype of Djelfa (2016) showed the lowest value (74 mg/100 g).

**Table 5.** Sterol Composition of the Atlas Pistachio Fruit in Different Geographic Zones in Algeria for Two Years (2015 and 2016).

Phytosterol Composition (mg/100 g)		Bechar		Batna		Djelfa	
		2015	2016	2015	2016	2015	2016
Demethylphytosterols (DMS)	$\beta$ -Sitosterol	81.4	75.2	76.1	-	80.4	74.0
	Campesterol	9.9	4.2	4.1	-	8.4	3.5
	Stigmasterol	3.0	2.0	2.1	-	5.0	3.6
	$\delta^7$ -Avenasterol	8.4	7.4	7.1	-	9.8	11.2
	Cholesterol	-	0.5	-	-	-	-
	Total	102.6	89.4	89.4	-	103.8	92.4
Methylphytosterols (MS)	Citrostadienol	1.9	1.9	1.8	-	2.0	2.1
	Total	1.9	1.9	1.8	-	2.0	2.1
Dimethylphytosterols (DiMS)	Cycloartenol	7.9	7.6	8.4	-	9.6	8.4
	Methylene cycloartanol	4.4	4.0	4.1	-	1.7	2.6
	Total	12.3	11.6	12.4	-	11.3	10.9
Total Phytosterols		116.8	102.8	104.4	-	117.1	105.5





**Figure 3.** Chromatogram of Phytosterols from Atlas Pistachio Seeds.

#### 4. Discussion

The vegetable oils are triglycerides that can be used for medicinal purposes, whose composition and content depend on the genetic variability and agro-environmental factors [26].

Indeed, the lack of either of the two fatty acids leads to poor health and causes deficiency symptoms. In addition, several studies have positively correlated essential fatty acid (EFA) intake with the reduction of cardiovascular, inflammatory diseases, obesity, and cancer [27–29].

In this study, the average oil content in *P. atlantica* Desf. seeds is 40%. These results were confirmed by Labdelli et al. [22] in other regions in Algeria.

Moreover, the different parts of the seed of Atlas pistachio are very rich in unsaturated fatty acids which have a favorable and beneficial effect on health in preventing coronary heart disease [29,30]. It should be noted that the seeds from Djelfa and Batna contained more PUFAs than those from Bechar. Generally, this result can be explained by the fact that the PUFA contents decrease in hot weather conditions. The presence of monounsaturated fatty acids, such as oleic acid, is of great importance because of their nutritional implications and their effect on the oxidative stability of oils [31].

A high amount of oleic and linoleic acids protects against certain metabolic disorders, cardiovascular diseases, and cancer [32,33].

It has been reported that MUFA can reduce low-density lipoprotein (LDL) cholesterol, while they can potentially increase HDL cholesterol [34]. The two main polyunsaturated fatty acids (PUFA) are linoleic acid (LA) and  $\alpha$ -linolenic acid (ALA), precursors of the fatty acid families “ $\omega$ -6 and  $\omega$ -3”, respectively, which are essential and specific fatty acids for health. They may play a role in reducing inflammatory diseases, such as rheumatoid arthritis, obstructive cardiovascular disease, diabetes, osteoporosis, and certain disorders such as Alzheimer’s disease [3,35].

The high ratio of unsaturated fatty acids to saturated fatty acids (MUFA + PUFA)/SFA in these seeds of *Pistacia atlantica* Desf. subsp. *atlantica* confirms their high nutritive value of activity and provides good oxidation prevention to oils [31,36]. However, it seems that this ratio was much lower than those of olive oil (4.8) and sunflower oil (6.75) [31]. According to current dietary guidelines for a healthy diet, the ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) above 1.5 is associated with good health [37].

The observed differences between the various parts of the fruits from all ecotypes could be due to genetic and environmental factors. In fact, our fruits were from different locations in Algeria which are characterized by different environmental conditions, such as temperature

and rainfall (Table 1). This specie has shown inter- and intra-population genetic diversity under geographical and environmental conditions in different areas in Algeria [38].

Moreover, Avato and Tava [12] demonstrated that biochemical composition depends on the studied section of plant. Our findings support the numerous research studies on plant oils of different species that have highlighted the impact of environmental factors, as well as storage conditions and duration on their quality and fatty acids composition [18,21,26,39,40].

The available findings indicated that the oxidative stability of these parts of the fruit could be used for the protection of vegetable oils against oxidative degradation [41]. The oxidizing index indicates the sensitivity of the oil to oxidation. A similar result has been reported in *P. atlantica* Desf. growing wild in Iran by Hazrati et al. [42] (2.79) and Tavakoli et al. [43] (4.23), showing that they can be considered as sources of stable oils.

Oxidation is one of the most common features that determine oil quality deterioration. The fatty acid composition as well as the antioxidant content of oils are the main factors that control this parameter [44]. The process occurs through rancidity resulting from oxidation that takes place at the double bond sites in the triacylglycerol molecules [45]. The degree of unsaturation of fatty acids plays an important role in the susceptibility of lipid systems to oxidation [45]. The variation of stability often depends on the genotype studied [46]. For instance, the oxidative stability of sesame oil depends on the moisture content of the seed, roasting duration, and temperature [47].

Generally, there is usually a reverse relationship between the PUFA/SFA ratio and the oxidizability value from edible oils and their oxidative stability [43]. According to the obtained results, it seems that *P. atlantica* Desf. contains oil with an appropriate oxidative stability [42].

The results of the present study are consistent with those obtained by Ben Ahmed et al. [1] and Salhi et al. [48]. These authors have shown that  $\beta$ -sitosterol, campesterol, and stigmasterol are the major components of the sterol fraction.

The content and composition of phytosterols vary according to plant species, varieties, and even environmental factors [21].

Through the activity of methyltransferase 2 (SMT-2), it may be possible to modify the levels and composition of sterols and fatty acids through the management of cultural practices. The observed impact on sterols and fatty acids composition could be explained by the fact that cytoplasmic acetyl-CoA is required for the synthesis of both fatty acids and phytosterols [15,21].

The concentration of phytosterols in the oils shows inter- and intraspecific variability. These variations are a function of environmental effects during grain filling on the dynamics of oil and phytosterol accumulation in sunflowers [49]. The increase in intercepted solar radiation (ISR) by plants not only increases the amount of oil but also the amount of phytosterols [49].

## 5. Conclusions

The fruits of the Atlas pistachio could be considered as a species that competes with oilseeds for its high oil content (>40%) and its biochemical composition. Therefore, the presence of a high percentage of unsaturated fatty acid, particularly oleic and linoleic acids and phytosterols compound, gives Atlas pistachio fruits, including the kernel and epicarp, great nutritional and industrial importance, especially in the pharmaceutical industry. Nevertheless, these characteristics depend on the genotype and environment conditions. Therefore, it is indicated that this tree be exploited by selecting the best seed sources or provenances.

A better knowledge of the compositional properties of seeds could help in the industrial application of this species. Data on the chemical composition of fruit should be used for educational purposes and for compiling composition tables of local foods.

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